SDS-Polyacrylamide gel electrophoresis

Proteins are separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to their molecular masses. 15% acrylamide gels are used in combination with a stacking gel. See Table 12.

Table 12: SDS-Polyacrylamide gel electrophoresis gels and buffers

Name	Components
5xSDS sample buffer	80 mM Tris pH 6.8 10 % SDS 12.5 % Glycerin 4 % (v/v) 2-Mercaptoethanol 0.2 % (w/v) Bromophenol blue
10x SDS-loading buffer	62 g Tris 20 g SDS 288 g Glycerol Fill to 2 L with dH2O
stacking gel buffer	1.5 M Tris-HCl pH 8.8
separation gel buffer	0.25 M Tris-HCl pH 6.8
Coomassie staining solution	1 g Coomassie brilliant blue R250 500 mL Ethanol 96 % denatured 100 mL Glacial acetic acid Fill to 1 L with dH2O
separation gel	380 mM separation gel buffer 12 % Acrylamide (37,5:1), 0.1 % SDS 0.05 % APS 0.05 % TEMED
stacking gel	125 mM stacking gel buffer, 5 % Acrylamide (37,5:1) 0.1 % SDS, 0.05 % APS 0.17 % TEMED

- SDS-PAGE-Samples are taken from different stages of the protein expression and purification process. Before induction and after induction samples are taken for an OD600 = 1.0 for the sample. The sample volume to be taken from the culture is calculated with the following equation:
  $V_{sample} = 1000 / OD_{600\ cultures}$

- The sample is centrifuged (60 sec, 13,300 rpm) and the pellet is resuspended in 60 µL Milli-Q H2O. Table 13 lists all samples used for SDS-PAGE analysis. All samples are mixed with 5 x SDS sample buffer and boiled at 96 °C for 10 min. 10 µL of each sample is loaded onto the gel. Electrophoresis is performed between 80 V (stacking gel) and 180 V (running gel) with SDS running buffer. Gels were stained with coomassie staining solution for 30 min at RT and destained with water overnight at RT prior to gel documentation. The PageRulerTM Prestained Ladder Mix (Thermo Fischer Scientific) is used as standard.